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INHALATION TOXICITY STUDIES ON CIGARETTE SMOKE
III. TOBACCO SMOKE INHALATION DOSIMETRY STUDY ON RATS

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SUMMARY

Smoke inhalation dosimetry studies have been carried out on rats, using a new exposure system. A range of cigarettes, tobacco types and smoke concentrations was used.

Penetration of smoke into the lungs was clearly demonstrated, and loads of total particulate matter (TPM) of up 1 mg were detected in the lower respiratory system of rats. The mass of TPM deposited was affected by the smoke concentration during exposure.

Deposition of TPM in the head of the rat was low in relation to total respiratory system deposition. A pattern of predominantly lung deposition was achieved under the conditions used for this series of experiments. This pattern was not affected by changes in smoke dilution level, cigarette or tobacco type.

INTRODUCTION

The rat is currently being used extensively in a number of laboratories carrying out inhalation toxicity studies with tobacco smoke. The work reported in this paper is concerned with particulate deposition in rats subjected to smoke in a new inhalation machine.

The aim of the work was to determine particulate deposition levels in rats subjected to diluted smoke under varying conditions. Some of the variables which were examined in this series of experiments included the effect of

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Abbreviations: COHb, carboxyhaemoglobin; DCBP, decachlorobiphenyl; DDD, p,p'-dichlorodiphenyldichloroethane; TPM, total particulate matter.

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cigarette and tobacco type, smoke dilution level and the sex of the animal on the deposition of smoke particulates in the respiratory system.

Although a limited amount of information on dosimetry is beginning to emerge, more basic work needs to be done on what is now considered to be an increasingly important aspect of inhalation toxicity studies with tobacco smoke. At this stage, the fairly extensive data presented below added to our present knowledge on smoke particulate deposition in the rat, provide a background for further studies and for comparison of data from other laboratories.

MATERIALS AND METHODS

The exposure system used has been described in detail elsewhere [1]. During exposure of animals smoke was generated under standard conditions and diluted to the desired level before passing to the exposure chamber of the inhalation machine. Diluted smoke filled the chamber throughout the whole of the exposure period.

Rats were obtained from Anglia Laboratory Animals (Wistar derived, strain CFHB) and maintained using conventional procedures. During exposure animals were fitted in restraining tubes, usually in groups of three, around the central exposure chamber.

The cigarette types used, their general characteristics and the exposure conditions selected are shown in Table I. Smoke dilutions were selected at particular levels based on a simple proportion of whole fresh smoke to air. The levels shown in the table are calculated from actual settings of flow meters on the exposure system during dosimetry runs. The cigarettes used covered a fairly wide range of delivery of total particulate material. Plain and filter types were used, and cigarettes were made from two basic tobacco blends.

Animals were allocated to the various experimental groups and, for all except one group, were subjected to a smoke acclimatisation period prior to an exposure to smoke from cigarettes spiked with the particulate phase marker.

During acclimatisation, the duration of exposure was increased gradually over 5 days until animals were subjected twice daily to the exposure regime detailed in Table I for each cigarette type. This full exposure schedule was given on successive days and on the 3rd day an exposure to smoke containing the particulate marker was carried out.

Assessment of total particulate material deposition in animals was carried out using techniques based on the use of DCBP as a particulate phase marker [2]. Cigarettes were injected with DCBP (approx. 2.2 mg/cigarette) and suitably conditioned before being used. For all cigarettes, the transfer of the particulate phase marker to mainstream smoke and DCBP/TPM ratios were determined. Following exposure to "spiked" cigarettes, animals were rapidly killed and tissues removed for analysis of DCBP, and hence TPM, retained in the various regions of the respiratory system.

The respiratory system was divided into 5 regions: (*1*) skinned head (less

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TABLE I

CIGARETTE TYPES AND EXPOSURE CONDITIONS USED FOR DOSIMETRY STUDIES ON RATS

Cigarette	Description	TPM delivery to exposure chamber (mg/cig.)	CO delivery (mg/cig.)	Smoke dilution used (smoke/air v/v)	Chamber smoke concentration (mg/l)	Exposure [a] time (min)
A	70 mm, plain, flue-cured, all lamina	37.6	19.9	1 in 14.0	7.7	10
B	85 mm (with 15 mm tip) flue-cured Virginia blend	20.2	14.9	1 in 11.4	5.6	9
C	85 mm (with 21 mm tip) U.S. blend	22.6	17.2	1 in 5.4	13.4	9
				1 in 9.8	7.3	
				1 in 28.5	2.5	
D	85 mm (with 21 mm tip) Modified U.S. blend	18.7	15.3	1 in 9.8	6.8	8

[a] Equals nearest whole puff number to standard butt length of 23 mm.

brain), (2) skinned lower jaw and tongue, (3) larynx, (4) trachea and (5) main bronchi and lungs. Samples were placed in pre-weighed jars and stored under deep-freeze conditions pending analysis.

Tissue samples were cut into small sections and extracted for 3 hours with refluxing cyclohexane in Soxhlet thimbles containing sodium sulphate. The crude extracts were concentrated on a Büchi evaporator then applied to individual columns of alumina (Activity grade super 1). The columns were developed with cyclohexane which was discarded and the DCBP-containing fraction was eluted with ether in cyclohexane (15% v/v).

The eluate was concentrated to small volume under vacuum, made up to a standard volume (5, 10 or 25 ml) with added DDD as internal standard. Aliquots (1 μl) of these solutions were analysed for DCBP using a Perkin Elmer Model F17 GLC fitted with dual Ni^{63} ECD at 300°C. The glass column (2 m × 3.0 mm i.d.) packed with 3.8% OV-1 on 80-100 mesh Chromosorb W (HP), was maintained at 260°C.

The DCBP was determined by a comparison with a calibration curve prepared from a series of standard solutions.

COHb levels in blood samples taken immediately following exposure were determined using an IL CO-Oximeter (Instrumentation Laboratories Limited).

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Animals subjected to smoke from cigarette A, were not acclimatised before exposure to DCBP-tagged smoke was carried out.

The effect of varying smoke dilution on TPM deposition and pattern of distribution of TPM in the respiratory system was carried out using cigarette C.

RESULTS

Raw data derived from animals exposed to smoke from each of the cigarettes used for this work are shown in Table II where deposition in the head and lower jaw samples has been combined to give total head deposition. Conditions varied to some extent for each of the series of exposures and detailed comparison of the data in this form is not particularly useful. However, the results do raise several interesting points which are worth commenting upon at this stage.

COHb levels were very similar in all groups of animals with mean values ranging from 25.7% to 33.0% COHb. Animals clearly did inhale smoke vapour phase and DCBP determinations confirmed that smoke particulate material was deposited in all parts of the respiratory system. Without exception, TPM load was greater in the lungs than in any other part of the respiratory system of the animals. Compared with the level of particulate deposition in the complete lower respiratory system, the mass of TPM deposited in the head of exposed rats was modest. Under the exposure conditions used for this part of the work, TPM deposition levels were within the approximate range of 350 μg–850 μg/animal. TPM retention per animal for the tissues examined was calculated at from 0.18% to 0.37% of the mainstream TPM which passed through the exposure chamber.

The calculation of percentage distribution of TPM detected in the various tissue samples analysed is shown in Table III. For all animals examined in this series, the largest proportion of TPM was found in the lower respiratory system. A mean of 17.9% of total TPM was detected in the head of one group of rats. For the remaining three groups, taking combined data for male and female animals, less than 10% of TPM was recovered from the complete head. The distribution of TPM marker in the head of these rats was low, when comparison is made with figures obtained for other laboratory rodents [3].

The animals exposed to smoke from cigarette A showed the highest per cent deposition of particulates in the head. Rats in this group were exposed without prior acclimatisation to smoke from high delivery, untipped cigarettes at the highest chamber smoke concentration used in this part of the study. Later (Table V), it is shown that low deposition of TPM in the head occurred in rats exposed to an even higher smoke concentration than that to which cigarette A animals were subjected. Whether the process of acclimatisation to smoke or filtration of mainstream smoke affects the deposition pattern in rats is not yet known. There was no obvious effect on deposition pattern due to exposure to smoke from distinctly different types of tobacco.

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TABLE II

DEPOSITION OF TPM IN RATS EXPOSED TO SMOKE FROM FOUR DIFFERENT CIGARETTES

Figures shown are mean and (S.D.).

Cigarette	Smoke dilution	Number and sex of animals	Mean body weight (g)	Mean TPM deposited (with S.D.)				Total respiratory [a] system TPM (μg)	Mean blood COHb (%)
				Head (μg)	Larynx (μg)	Trachea (μg)	Lungs (μg)		
A	1 in 14.0	6 Male	214	147.6 (37.6)	47.9 (48.8)	34.8 (19.5)	596.1 (254.2)	826.3 (254.3)	26.5 (6.1)
B	1 in 11.4	9 Female	197	64.2 (34.3)	31.0 (27.6)	21.3 (12.2)	647.3 (195.8)	763.8 (226.4)	26.1 (6.5)
C	1 in 9.8	6 Female	192	36.5 (20.2)	7.9 (3.9)	6.7 (5.4)	540.0 (203.2)	591.1 (202.3)	32.7 (6.8)
C		6 Male	216	43.4 (20.9)	10.6 (4.8)	5.4 (11.5)	618.8 (193.8)	678.2 (197.0)	33.0 (6.2)
C Pooled data	1 in 9.8	12	204	40.0 (20.8)	9.3 (4.6)	6.0 (4.0)	579.4 (202.4)	634.7 (204.4)	32.9 (6.5)
D	1 in 9.8	6 Female	186	35.0 (17.3)	5.3 (2.0)	7.3 (3.8)	318.7 (193.5)	366.3 (207.0)	26.8 (6.3)
D		6 Male	225	16.4 (7.9)	7.7 (6.1)	7.6 (4.3)	393.6 (122.1)	425.3 (133.8)	25.7 (5.7)
D Pooled data	1 in 9.8	12	206	25.7 (16.3)	6.5 (4.7)	7.5 (4.1)	356.2 (166.1)	395.9 (176.8)	26.3 (6.1)

[a] Head, larynx, trachea and lungs.

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TABLE III

MEAN PERCENTAGE DISTRIBUTION OF TPM IN WHOLE RESPIRATORY TRACT OF RATS EXPOSED TO SMOKE FROM FOUR DIFFERENT CIGARETTES

Cigarette	Smoke dilution	Number and sex of animals	Mean body weight (g)	Percentage distribution of TPM			
				Head (%)	Larynx (%)	Trachea (%)	Lungs (%)
A	1 in 14.0	6 Male	214	17.9	5.8	4.2	72.1
B	1 in 11.4	9 Female	197	8.4	4.1	2.8	84.7
C	1 in 9.8	6 Female	192	6.1	1.9	1.1	90.9
		6 Male	216	6.4	1.5	0.9	91.2
Pooled data	1 in 9.8	12	204	6.3	1.7	1.0	91.1
D	1 in 9.8	6 Female	186	9.6	1.5	2.0	87.0
		6 Male	225	3.9	1.8	1.8	92.6
D Pooled data	1 in 9.8	12	206	6.8	1.7	1.9	89.8

TPM was detected in substantial amounts in the lungs of animals. It follows that TPM dose for the various regions, expressed as μg TPM/g of tissue (Table IV), should be heaviest in the lungs and relatively low in the other regions of the lower respiratory system. There was no evidence, using this method of expressing dose, of particularly high deposition of TPM in either the larynx or trachea of rats.

Where direct comparisons could be made (cigarettes C and D) statistical analysis did not show any significant differences between sexes in either mass TPM deposition or TPM dose/g tissue (Table IV) to any region of the respiratory system.

To assess the effects of differences in smoke concentration on deposition characteristics, pre-acclimatised animals were subjected to smoke from cigarette C over a wide range of concentrations. Chamber concentrations of smoke at the dilutions used are shown for each group of animals, together with appropriate dosimetry data, in Table V.

As smoke concentrations in the exposure chamber increased, TPM mass deposited in the animals and blood COHb levels increased. Despite the changes in mass TPM deposition, the overall deposition pattern remained the same. The largest proportion of TPM reached the lower respiratory

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TABLE IV

DEPOSITION OF TPM PER UNIT WEIGHT OF TISSUE IN RATS EXPOSED TO SMOKE FROM FOUR DIFFERENT CIGARETTES

Figures shown are mean and (S.D.)

Cigarette	Smoke dilution	Number and sex of animals	Mean body weight (g)	Larynx (μg/g)	Trachea (μg/g)	Lungs (μg/g)	Complete lower respiratory system (μg/g)
A	1 in 14.0	6 Male	214	442.9 (360.0)	310.6 (119.0)	549.2 (290.7)	515.2 (250.2)
B	1 in 11.4	9 Female	197	447.7 (389.5)	399.6 (272.8)	601.9 (189.1)	583.4 (185.6)
C	1 in 9.8	6 Female	192	102.6 (33.4)	99.8 (67.8)	464.7 (170.4)	424.4 (155.1)
		6 Male	216	133.0 (57.7)	84.3 (27.5)	521.9 (159.5)	475.7 (143.8)
C Pooled data	1 in 9.8	12	204	117.8 (49.5)	92.1 (52.3)	493.3 (167.8)	450.1 (151.7)
D	1 in 9.8	6 Female	186	75.1 (26.6)	136.8 (61.1)	294.0 (175.4)	274.1 (161.6)
		6 Male	225	106.8 (90.1)	125.7 (76.9)	334.9 (121.3)	311.2 (114.4)
D Pooled data	1 in 9.8	12	206	90.7 (68.4)	131.3 (69.7)	314.5 (152.1)	292.7 (139.3)

region, particularly the lungs, and only a relatively small proportion deposited in the head. Statistical analysis revealed no significant differences in percentage deposition level at the three smoke concentration levels, although percentage deposition in the lungs did tend to fall and in the head to increase slightly as the concentration of smoke increased in the exposure chamber.

It is interesting to relate TPM deposition in the various respiratory regions to smoke concentration levels experienced by the three groups of rats exposed to smoke from cigarette C. In Table VI, the values for mean TPM dose to each of the various respiratory regions are expressed as ratios related to the corresponding TPM level found after exposure at the lowest smoke concentration. Similarly, smoke concentrations are expressed as ratios relative to lowest concentration i.e. at the dilution level 1 in 28.5, smoke : air.

The ratios of total deposition of TPM in rats, and of deposition of TPM in the lower respiratory system, lungs and larynx were very similar though slightly lower than the smoke concentration ratios for the different smoke dilutions. The trachea and head deposition ratios did not match smoke concentration ratios quite so closely.

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TABLE V

DEPOSITION OF TPM IN MALE RATS EXPOSED TO SMOKE FROM CIGARETTE "C" AT DIFFERENT CONCENTRATIONS

Figures shown are group mass mean deposition (S.D.) and mean % deposition

Chamber TPM concentration (mg/l)	Number of animals	Mean body weight (g)	TPM deposited						Mean blood COHb (%)
			Head (μg) (%)	Larynx (μg) (%)	Trachea (μg) (%)	Lungs (μg) (%)	Lower [a] respiratory system (μg)	Total respiratory system TPM (μg)	
2.5	6	223	11.5 (2.9) 4.6	4.2 (2.7) 1.7	2.4 (1.2) 1.0	233.5 (102.4) 92.8	240.1	251.6 (100.7)	19.0 (1.6)
7.3	6	216	43.4 (20.9) 6.4	10.6 (4.8) 1.5	5.4 (1.5) 0.9	618.8 (193.8) 91.2	634.8	678.2 (197.0)	33.0 (6.2)
13.4	4	223	94.2 (50.0) 8.4	19.8 (9.6) 1.8	16.7 (12.8) 1.5	994.5 (287.5) 88.4	1031.0	1108.3 (311.8)	46.0 (6.1)

[a] Sum of means of larynx, trachea and lungs.

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TABLE VI

THE RATIOS OF SMOKE CONCENTRATIONS AND MASS TPM DEPOSITED IN VARIOUS REGIONS OF THE RESPIRATORY SYSTEM OF RATS EXPOSED TO SMOKE FROM CIGARETTE "C" OVER A RANGE OF DILUTIONS

Dilution level (smoke/air v/v)	Smoke concentration ratio	Head	Larynx	Trachea	Lung	Lower respiratory system	Total TPM deposited
1 in 28.5	1	1	1	1	1	1	1
1 in 9.8	2.92	3.77	2.52	2.25	2.65	2.64	2.70
1 in 5.3	5.36	8.19	4.71	6.96	4.26	4.29	4.41

DISCUSSION

Analyses of respiratory system tissues confirmed that smoke particulate material effectively penetrated to the lungs of rats exposed on the new exposure system used for this work. Without exception, for a particular exposure situation TPM deposition was always highest in the lung, in comparison with the larynx, trachea and head of the rat.

Deposition in the head of rats in all groups, irrespective of cigarette type, restraining tube or smoke concentration used, was relatively low. The highest head deposition of TPM, expressed as a percentage of TPM in the complete respiratory system, was 17.9%. In other groups of animals TPM deposition in the head was consistently less than 10%. This finding is of interest in relation to other figures published in the literature for the rat and other rodent species [3], because it contradicts the view [4] that filtration of TPM in the nasal region of small laboratory animals is a major obstacle to the development of inhalation toxicity studies with cigarette smoke.

TPM deposition in the head was highest in those rats which had not been acclimatised before being exposed to smoke from "spiked" cigarettes. Acclimatised rats in other groups exposed at similar concentrations of smoke showed a much lower proportion of TPM in the head. Although no firm conclusions can be drawn from the comparison of data from the different groups, the contrast in deposition pattern is striking and the question of acclimatisation should be investigated more fully.

Information in this study relates to samples from 45 rats only. Analysis of a total of around 80 rats, including data not recorded in this paper, give a consistent picture of a low percentage TPM deposition in the head. The data shown here are based on analyses carried out following careful, prolonged and repeated extraction of samples. As far as is known they represent accurately the actual and relative amounts of TPM in the various parts of the respiratory system of the exposed animals. The evidence presented here seems to show that for the rat *in our exposure system*, at least, nasal filtration was not a major obstacle to effective dosing of the lower respiratory

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system with smoke particulate material. It is significant that rats exposed under the conditions used in these dosimetry studies do show within a relatively short time consistent and quite marked changes in lower respiratory system pathology. This important aspect of our work will be reported in detail as soon as possible.

The difficulty of comparing inhalation studies carried out in different laboratories, using different exposure systems, has been stressed before. Figures presented here can be taken as relevant only to the exposure system and conditions used for our work. They can not be used to draw conclusions about dosimetry characteristics of rats in general, or for animals exposed under a variety of conditions on different smoking machines.

This qualification is perhaps re-inforced by recent work [5] on the Syrian golden hamster. In animals exposed in one system ("closed") TPM deposition in the head was 26.3% of total respiratory system load. For animals exposed in a second system (Hamburg II) the head deposition was almost halved, at 14.7% of a reduced total respiratory system load. Whatever the reason for the shift in deposition pattern, this experiment illustrates clearly that TPM mass deposition and distribution pattern may be affected by the exposure system used.

For the range of smoke dilutions reported here, TPM load to the lower respiratory system of animals ranged from a mean of 0.29 mg to 1.03 mg TPM per animal. A lung TPM dose of approx. 1 mg was associated with a high mean COHb level of 46.0%. In our system, for a regime of 2 exposures per day separated by an interval of 4 h, the upper value of approx. 1 mg TPM per session, appears to be close to the maximum TPM dose which animals will tolerate reasonably well following a short acclimatisation period. Whether this dose level could be increased substantially with a more prolonged acclimatisation of animals has not yet been determined.

Over the range of smoke dilutions which can probably be most usefully used for long-term inhalation toxicity studies (1 in 5 to 1 in 30 smoke : air, v/v) mass TPM deposition in the respiratory system, but not the distribution pattern, was affected by the chamber smoke concentration. TPM deposition increased as chamber concentrations increased, even at the highest concentrations used, when smoke was obviously markedly irritant to the rats. In the small experiment reported here, the increase in the ratio of TPM load to the various respiratory tissues tended to be less than the increase in ratio from lowest to higher smoke concentrations. More work is needed to clarify the effect of duration of exposure and smoke concentration on the actual dose of TPM retained by animals.

Mean levels of TPM retained in rats did follow smoke concentration gradient quite closely. However, it is apparent from calculated variance of means for all groups of rats, that the range of deposition of TPM in animals exposed under the same conditions was quite wide. For the lungs a two- to four-fold difference in TPM load between the highest and lowest dose was common in a group of, say, 6 rats. Similar variability in dose within groups of animals exposed under the same conditions has been shown for other species [3].

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